

INF1291/2.2 – Preparation of plasma to be sent to Molecular Diagnostics for fetal genotyping



Blood and Transplant

Copy No:

Effective date: 28/03/2023

Sample requirements:

16 mL EDTA blood from mother **per test requested**.

Buffy coat prepared from EDTA blood from father (optional): **only for RhD typing**

Request form requirements:

FRM4674 Request for Fetal Blood Group Genotyping from Maternal Blood

Health and Safety:

- Gloves and laboratory coat must be worn when handling samples.
- Use filtered pipette tips when preparing the plasma aliquots.
- Process the blood in a clean DNA free area of the laboratory. Non-invasive testing is extremely sensitive and contamination of fetal samples with any other DNA can lead to false positive results.

Procedure:

1. Maternal EDTA blood must be processed within **48 hours** of venepuncture for fetal Kell genotyping, within **72 hours** of venepuncture for fetal RhD/C/c/E genotyping.
2. Transfer blood sample to labelled 15mL plastic tube and centrifuge at 1942-2103xg for 10 minutes with the brake off.
3. Visually assess the appearance of the plasma layer and note if haemolysis has occurred. Haemolysed samples will not be tested.
4. Without disturbing the buffy coat, transfer plasma into a clean labelled 15mL plastic tube. Mix the plasma briefly by inversion, then transfer 1.4mL of plasma into 4x labelled 1.5mL tubes (minimum of 4x tubes per patient). Centrifuge the plasma aliquots at 4700xg for 10 minutes.
5. Remove 1.2mL of plasma from the 1.5ml plasma tubes to 4x new 1.5mL tubes, without disturbing pellet (minimum of 4x tubes per patient). **Label the plasma aliquots with three points of identification (i.e. full name, DOB and hospital number/unique identifier) and date separated** (see template below). Store at -20°C to -30°C.
6. Remove 700µl buffy coat from the 15mL tube into a clean 1.5mL tube. Label the buffy coat tube with three points of identification (**i.e. full name, DOB and hospital number/unique identifier**) and **date separated**. (see template below). Store at -20°C to -30°C.

<i>SURNAME</i>
<i>First name</i>
<i>DOB</i>
<i>Hospital No/unique ID</i>
<i>Separation Date</i>

- Send the request form, the frozen plasma aliquots and buffy coat on dry ice to Molecular Diagnostics, NHSBT, 500 North Bristol Park, Northway. Filton, Bristol, United Kingdom, BS34 7QH.
- Pack with sufficient dry ice to ensure the samples remain frozen for the duration of transport and that they arrive between 09:00-17:00, Monday to Friday. Samples must be received within 5 days of shipment. Samples that **do not arrive frozen will not** be tested.
- The **name of the person who prepared the plasma samples** and the **date of the plasma preparation must** be included on the request form.
- The **requester must sign** the request form to acknowledge that the NHSBT Terms and Conditions apply to this referral unless they have signed a contract with NHSBT.

Samples/ request forms that do not meet the acceptance criteria listed above will not be tested.